

**REMARKS****I. Status of the Claims**

In a Decision on Appeal No. 2001-0695, Order pursuant to 37 CFR § 1.14 (g) the Board reversed rejection of claims 1 through 6 and 9 through 12, directed to a method of making a chimeric ungulate under 35 USC §112, paragraph 1.

The Board affirmed rejection under 35 USC § 112, paragraph 1 of claims 15 through 20, directed to a method of isolating an embryonic stem cell culture. These claims are canceled.

**II. The Board Decision is that Claims 1-6 and 9-12 Are Enabled**

The two sets of claims were separated in the Appeal because they are very different in scope: "making a chimeric ungulate" (claims 1 through 6, and 9 through 12) is very different from "isolating and purifying an embryonic stem cell culture" (claims 15 through 20).

The Board agreed with appellant's position that there is no statutory requirement to provide working examples, and that enablement of claims 1 through 6 and 9 through 12 satisfied the *Wands* criteria.

On page 6 of the Decision, paragraph 3:

We agree that the examiner has not shown that the claims directed to a method of making chimeric animals are not enabled.

On page 7 the Board disagreed "We disagree" - that more than one working example was needed.

The Board agreed with appellant's position that germ line transmission of embryonic stem cells was not required to produce *chimeric ungulates*, so that the argument about totipotent cells was not relevant for claims 1 through 6 and 9 through 12. Despite the Board decision, the examiner is now trying to apply Board comments about claims 15-20 to claim 3, which is in the

group of claims 1 through 6, thereby negating the Board's reversal of claims 1 through 6 and 9 through 12.

In a lengthy opinion which is to be published, the Board clearly understood the claims, including dependent claims, and understood and addressed the arguments. It is presumptuous to assume that with all the discussion of "pluripotent" vs. "totipotent", and distinguishing the two sets of claims with respect to that argument, that the Board failed to notice that claim 3 - a short claim near the top, contained the word "totipotent". The statement on page 4-5 of the Office Action is not true that the Board "clearly" found claim 3 unpredictable and there is no proof that only claim 1 was considered.

On pages 7 through 9 of the Decision the Board noted that totipotent cells and transgenic animals are preferred but not required. The focus is on making chimeric animals for which pluripotent and totipotent cells are suitable.

On page 9, paragraph 1 the Board decided "The rejection of claims 1 through 6 and 9 through 12 is reversed".

The Board never said totipotent cells were not enabled even for claims 15 through 20, a section of the decision the examiner now wants to apply to claim 3. The Board didn't dispute enablement isolating of porcine ES cells - the rejection was to breadth including all ungulates. The discussion regarding "pluripotent or totipotent" for claims 15 through 20 was under "Other Issues" and this is clearly not the basis of the Board decision to maintain rejection of 15 through 20.

Examiner seems to take the position that **only** totipotent stem cells would be "true" embryonic stem cells sufficient to satisfy the requirements of claims 15-20. This position appears to be in error (*emphasis added*).

**Although we disagree with this aspect of the examiner' reasoning**, we agree with his ultimate conclusion that the claims (*emphasis added*) are not fully enabled, as discussed in detail above.

### **III. Kashiwazaki was Previously Considered**

In the record, Kashiwazaki was removed as a basis for rejection by amending claims by adding "cultured" to modify ES cells (September 16, 1997, pages 1, 2, 4-7, 9, 11-12 of the Office Action). There is no reason to reintroduce this rejection.

### **IV. Evans is Not a 102 Bar**

Claims are novel and non-obvious in light of the cited art, and particularly PCT publication WO 90/03432 of Evans et al. Evans did not "make chimeric embryos." Furthermore, the "ES cells" referred to in the Evans publication are different from the "ES cells" of the present invention. Differences between the purported swine ES cells of Evans and those of the present invention are described in the present application. ES cells are defined on the basis of morphology as observed under certain conditions, the cell type being defined on the basis of morphology as observed under certain conditions, the cell type being defined at a stage of development and being predictive of totipotency. Such differentiation results from *in vitro* or *in vivo* conditions, but those of skill in the art would recognize "a nerve cell", for example, as distinct from the "ES cell" form which it originated. Even if the cells of Evans were cells at a different stage of development than those of the present invention, or cells cultured in different conditions, they are not shown to be "ES cells" as defined by their ability to produce tumors capable of differentiating into multiple cell types (which is the ultimate test of ES cells) to produce chimeric swine.

The disclosure in Evans does not provide an enabling disclosure of isolated embryonic stem cells for an ungulate. In fact, the morphology of the cells disclosed in Evans is not even

consistent with the morphology of embryonic stem cells according to the cells of the present invention. Accordingly, a skilled technician when faced with the problem of providing an isolated embryonic stem cell from an ungulate would not be taught from the disclosure of Evans to produce cells according to the present invention, since a skilled practitioner would not consider the cells of Evans to be embryonic stem cells. As can be seen from Appendix A citing to figures and tables the present application, the cells of the present application differ significantly in their morphological characteristics from those of Evans. In Appendix A, a declaration from Dr. Strelchenko, an expert in the field of embryonic stem cells, testifies that the cells taught by Evans are not the ES Cells of Wheeler (**Appendix A**). Dr. Strelchenko agrees with the tabulated differences between Wheeler and Evans that are also appended as Exhibits B-H.

#### **V. Clark Does Not Relate Elements of the Claims**

**Clark et al.** relate introducing "A fusion gene comprising  $\beta$ -lactoglobulin sequences and those encoding antihemophilic human factor IX" into sheep where it was reported to be "expressed in the mammary gland and the corresponding protein is secreted into milk," (Abstract). These are transgenic, not chimeric ungulates of claim 1, which as the Board of Appeals agreed, are completely different categories of animals. The gene was "microinjected into fertilized eggs" (p. 951, col. 1), there is no mention of embryonic stem cells as in claim 1.

The authors state:

"The only proven route for gene transfer in domestic livestock is by pronuclear injection."

p. 953, col. 1, and

"In the future, it is to be hoped that the successful development of embryonic stem (ES) cells from domestic livestock will facilitate gene transfer."

p. 954, col. 1

#### **VI. Other Issues**

A terminal disclaimer will be filed when claims are in condition for allowance.

Claims are not obvious. To properly combine two references to reach a conclusion of obviousness, there must be some teaching, suggestion or inference in either or both of the references, or knowledge generally available to one skilled in the art, which would have led one to combine the relevant teachings of the two references. *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc. et al.* (CAFC 1985) 776 F. 2d 281, 227 USPQ 657; *Ex parte Levengood, supra.* Both the suggestion to make the claimed composition or device or carry out the claimed process and the reasonable expectation of success must be founded in the prior art, not in applicant's disclosure. *In re Vaeck* (CAFC 1991) 947 F. 2d 488, 20 PQ. 2d 1438. Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious, *Ex parte Hiyamizu* (BPAI 1988) 10 PQ. 2d 1393, absent evidence of a motivating force which would impel persons skilled in the art to do what applicant has done. *Ex parte Levengood* (BPAI 1993) 28 PQ. 2d 1300. The references, viewed by themselves and not in retrospect, must suggest doing what applicant has done. *In re Shaffer* (CCPA 1956) 229 F. 2d 476, 108 USPQ 326; *In re Skoll* (CCPA 1975) 523 F. 2d 1392, 187 USPQ 481.

No fees are believed due at this time, however, please charge any additional deficiencies or credit any overpayments to deposit account number 12-0913 with reference to our attorney docket number (21459/90114).

Respectfully submitted,

A handwritten signature in cursive script, appearing to read "Alice O. Martin".

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October 3, 2003

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# APPENDIX A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.: 76645/115/BIRE

In re patent application of

Matthew B. Wheeler

Serial No.: 08/063,095

Group Art Uni: 1804

Filed: May 14, 1993

Examiner: Bruce Campell

For: TRANSGENIC SWINE COMPOSITIONS  
AND METHODS

## DECLARATION OF DR. NICK STRELCHENKO UNDER 37 C.F.R. § 1.132

Honorable Assistant Secretary and  
Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Dr. Nick Strelchenko, hereby declare that:

1. My relevant experience and background in the field of swine breeding and tissue culture are as follows:

Presently, I am Research Scientist, American Breeder Service.

I have published 18 journal articles on aspects of cell culture.

2. It has been represented to me that the attached figures and documents describing cells have been taken from a filed United States patent application on which Matthew B. Wheeler is named as the inventor and which has the title indicated in the caption of this Declaration. I will refer to this application as the "Wheeler application." These figures (Exhibit A) relate to porcine embryonic stem ("ES") cells in tissue culture and compare the appearance (morphology) of the ES cells of Wheeler with that of cells alleged in the Evans application (see Paragraph 3, below) to be porcine ES cells.

3. I have reviewed the International Application No. PCT/GB89/01103, International Publication No. WO 90/03432, which I will refer to as the "Evans application."

4. The descriptions of the ES cells in the Wheeler patent application as shown in Exhibits B-H are consistent with the corresponding FIGURES (Exhibit A) showing the appearance (morphology) of ES cells.

5. Based on my comparisons of the appearance of the ES cells of Wheeler with that of cells described in the Evans application, I agree with Dr. Wheeler's comparisons between mouse ES cells, the alleged "ES" cells of the Evans application, and ES cells of Wheeler described in the Wheeler application. There are morphological similarities between mouse ES cells and porcine ES cells of the Wheeler application, and differences between either of those cell types and the alleged ES cells described in the Evans application. Differences are in size, monolayered vs. multilayered growth, and ability to see distinct cells when in multilayers, using a light microscope.

6. The embryonic stem cells ("ES cells") produced according to methods of the Wheeler application and described therein, differ in morphological and growth characteristics from cells of the Evans application which are also purported to be porcine embryonic stem cells.

7. The morphology of cells described in the Wheeler application is consistent with that of embryonic stem cells; whereas, the cells described in the Evans application are not.

\* \* \* \*

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any application in connection with which this declaration is used or any patent issuing on any such application.

Respectfully submitted,

9/21/94  
Date

  
Dr. Nick Strelchenko



# EXHIBIT A

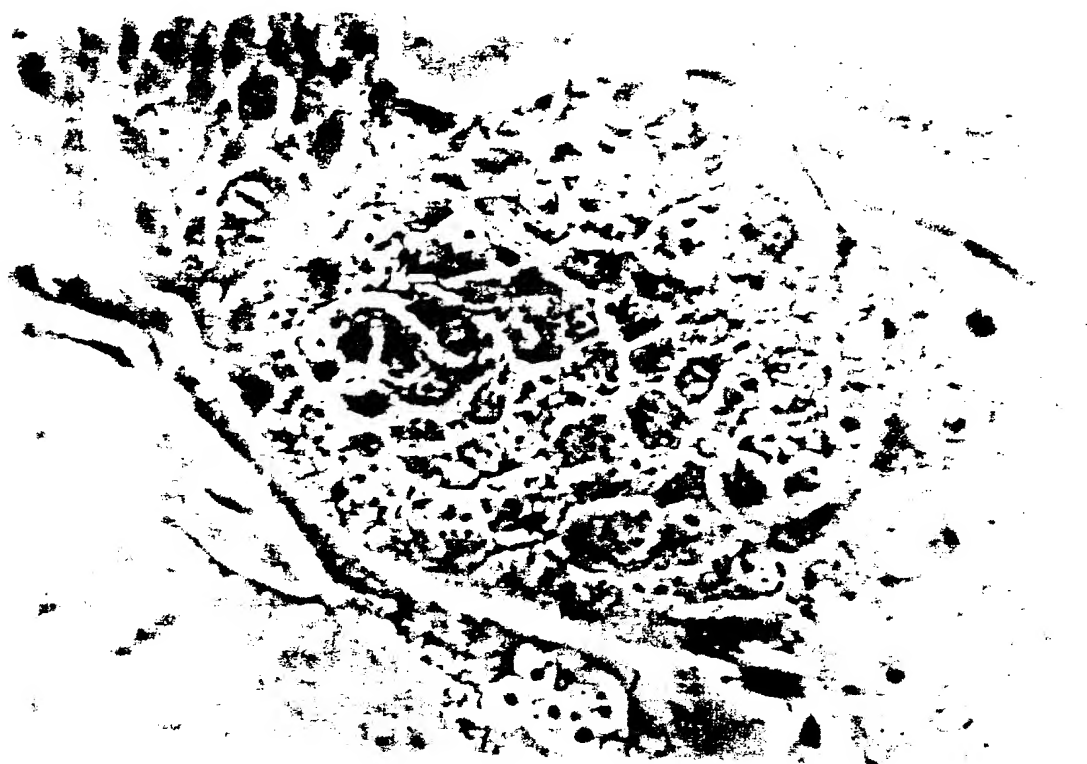


FIG. 1A

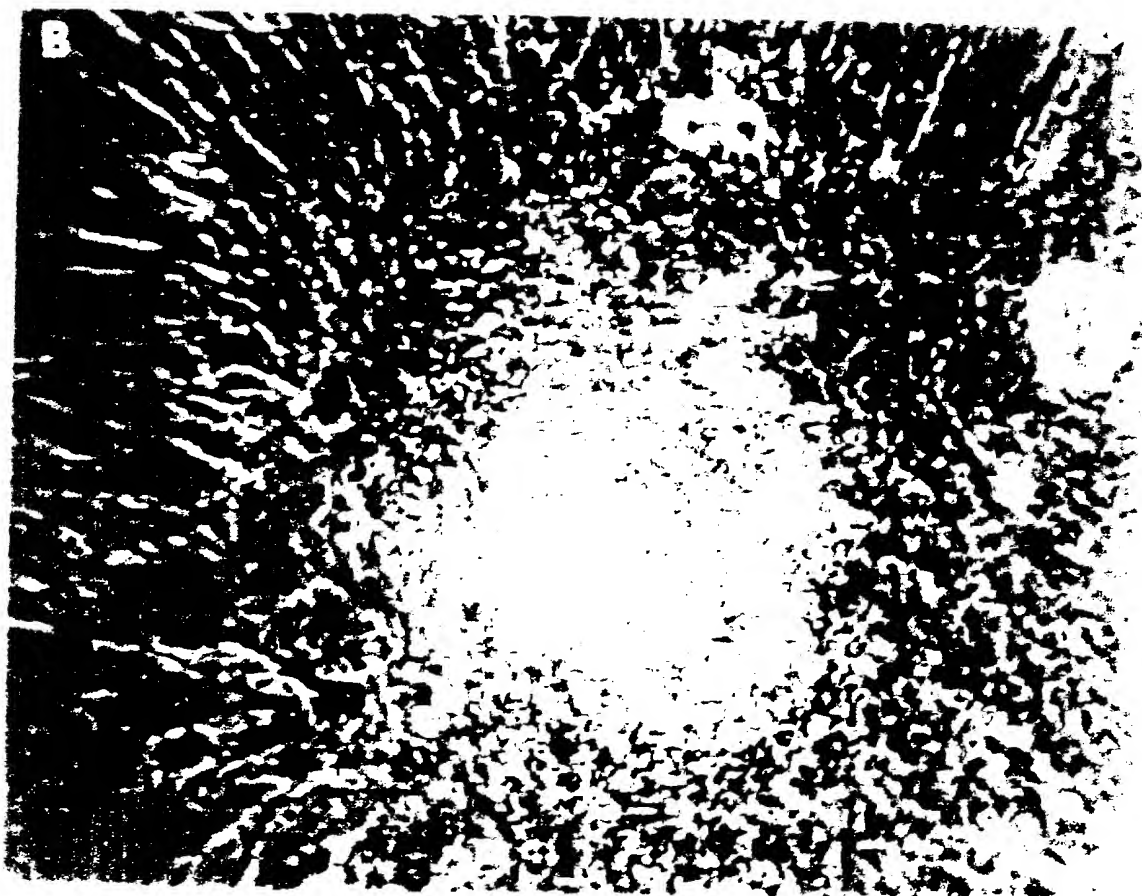


FIG. 1B

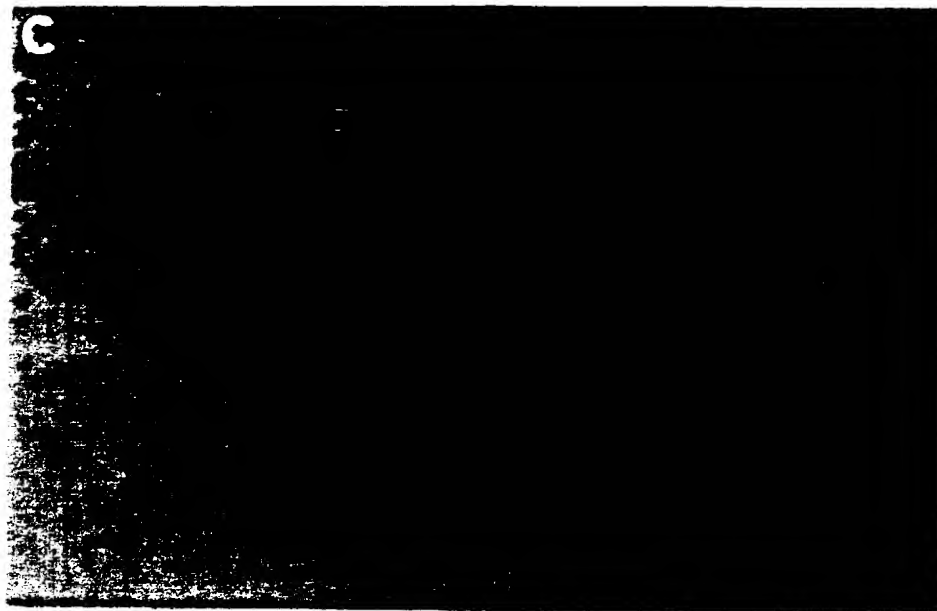


FIG. 1C

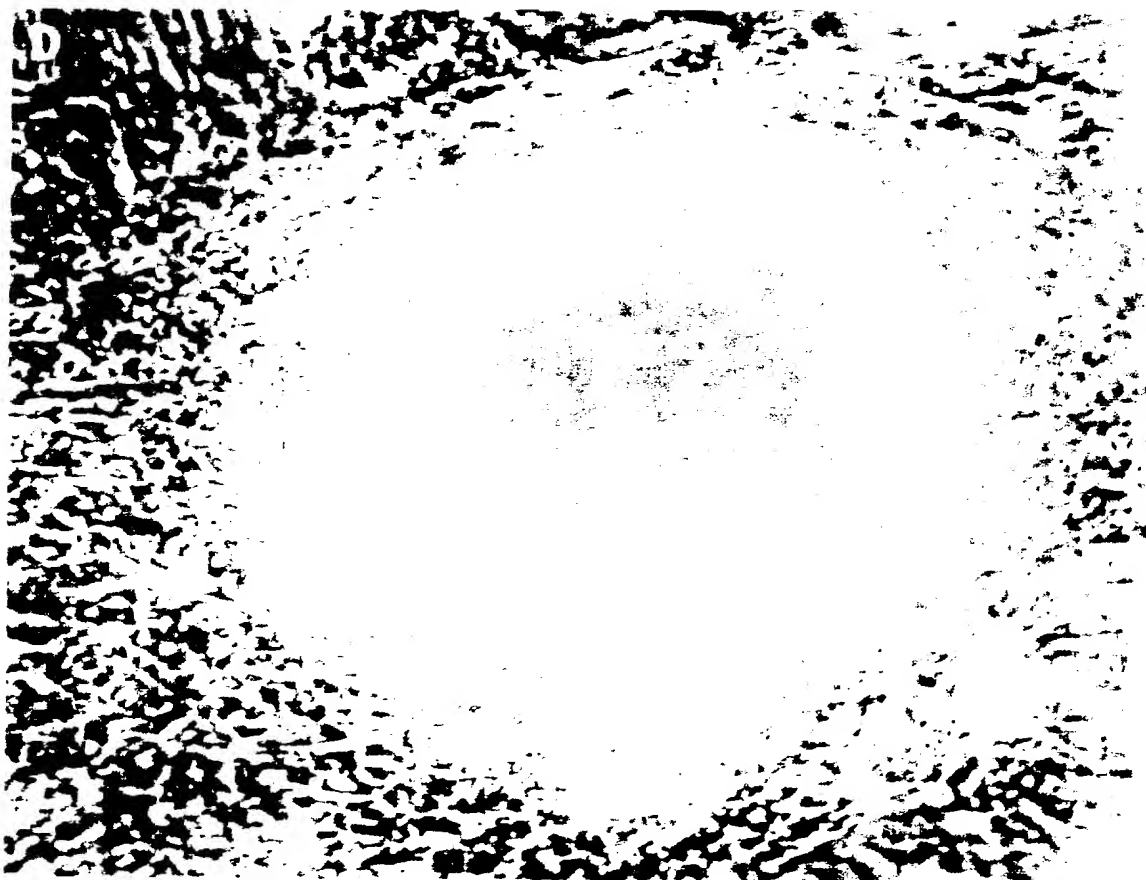


FIG. 1D



**FIG. 2**

## Exhibit B

### BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a comparison of morphological characteristics of development of cells designated "stem cells" by Evans (top panel) and the "embryonic stem cells of the present invention" (bottom panel).

(A) nest of undifferentiated cells in an established cell line at 250X magnification (FIG. 5A, Evans patent, photo is same as FIG. 3A from Notarianni et al., 1990, where this photo was taken from, i.e., FIG. 5A patent = FIG. 3A Notarianni et al., 1990);

(B) cluster nest of undifferentiated "embryonic stem cells" from an established cell line of the present invention at 200X magnification;

(C) monolayer growth of Evans undifferentiated cells (FIG. 5B, Evans); versus

(D) multilayered growth of the "embryonic stem cells" from an established cell line of the present invention at 200X magnification.

FIG. 2 ES cells of the present invention stained with Giemsa at 400X; cells are dispersed and fixed on slides.

## Exhibit C

### Description of Dr. Wheeler's ES Cells

As a preliminary scan for pluripotency of the ES cell lines, undifferentiated morphology is sought using the light microscope. Morphologically ES-cells are small (about 8-15 microns in diameter) and rounded, and possess large dark nuclei which contain one or more prominent nucleoli. The cytoplasmic to nuclear ratio is about 15:85, and the growth parameters comprise a doubling time of approximately 18-36 hours and multilayered rather than monolayered growth.

## Exhibit D

Changing media and subculturing are used to maintain healthy, cultures of the appropriate density, generally about  $1-2 \times 10^6$  cells/100 mm dish which contains about 10-12 ml of medium. The modal chromosomal count, that is, the number of chromosomes characteristic of the euploid pig genome, is 38.

## Exhibit E

NOTE: This step may occur at any point where ES cells of proper morphology are observed.

### Step 8:

Periodically it is necessary to pluck colonies as outlined above and re-isolate the ES cells with consistent morphology, size 8-15 $\mu$ , with a nuclear to cytoplasmic ratio of ~85:15, and growth characteristics (doubling time of 18-36h ).

NOTE: Maintenance of these isolated, purified undifferentiated ES cell lines is required to insure the proper cell type for generation of chimeras and for nuclear transfer. Some differentiation occurs spontaneously during *in vitro* culture and as a result of the freezing process. These differentiated cells do not subculture well, but occasionally it is necessary to re-purify the ES cells from the differentiated cells.

## Exhibit F

An initial step in the method is to establish a stable, undifferentiated embryonic stem (ES) cell line. For purposes of the present invention, stable means maintaining essentially similar cell types and growth parameters, through serial subcultures, under the same environmental conditions, and maintaining a stable, modal chromosome complement. "Modal" refers to the most frequent chromosomal count per cell. "Stable" in reference to a chromosomal complement refers to maintenance of the same modal number cell and culture morphology over repeated subculture. "Undifferentiated" in this context means not showing morphological or biochemical evidence of differentiation. An embryonic stem cell is an undifferentiated cell which is capable of differentiating into embryonic structures. An embryonic stem cell line is derived from a culture of embryonic stem cells.

## Exhibit G

### 2. *In Vitro* Characterization of ES-Cell Lines

An aspect of the invention is to select a transformed embryonic stem cell *in vitro* which is likely to produce a chimeric state when introduced into a pig embryo. The selection criteria are based on morphological characteristics of the transformed embryonic stem cell. Generally, morphological characteristics identifiable by inspection of the cell using the light microscope are predictive, although other assays for predictive morphological characteristics are also within the scope of the present invention.

Swine embryonic stem cells of the present invention are translucent, epithelial-like in appearance, and tend to form colonies or nests (clumps) of multilayers as opposed to monolayer growth. (FIGS. 1, 2). Cells have light refractory or round or polygonal cell borders. The cells will also form fluid filled domes with cells exhibiting the currently described ES morphology. The doubling rate of these cells is about 18-36 hours. These characteristics differ little from those reported for mouse embryonic stem cells, but do differ significantly from those reported by Evans. (FIG. 1).

Similarities of swine to mouse embryonic stem cells include that the nucleus to cytoplasmic ratio is approximately 85:15. The nucleus is round and contains several prominent nucleoli. Cell size varies somewhat lines isolated, but most of the stable lines consist of cells with diameters in the range of 8-15 microns.

In Table 2, the differences between the purported ES swine cells of Evans and those of the present invention are set forth. Also, the similarities between the swine cells disclosed herein and the ES cells of mice are described.

Table 2  
COMPARISON OF CELL MORPHOLOGY OF  
MICE AND OF SWINE ES CELLS

Parameter	Mice	Evans Swine	Swine of the Present Invention
Size	11-12 $\mu$ m	"larger 8-15 $\mu$ m than those of the mouse"	
Shape	round	round	round
Monolayer colonies	no	yes	no
Distinct individual cells can be identified	no	yes	no
Cytoplasm (% of cell vol)	25%	small	10-25%
Nucleus (% cell vol)	75%	large (no data)	75-90%
Number of nucleoli	2-4	2-4	2-4
Teratocarcinoma production in SCID mouse	yes <sup>b</sup>	?	yes
State of embryo cultured	3.5 d blasto- cysts	6.5-11 d hatched blastocysts	7.5-10 d hatched blastocysts

<sup>a</sup> Evans relates "a variety of sizes" (column 10 of Evans patent), and sizes varying among cell lines.

<sup>b</sup> Wheeler et al. unpublished results.

## Exhibit H

Swine embryonic stem cells of the present invention are translucent, epithelial-like in appearance, and tend to form colonies or nests (clumps) of multilayers as opposed to monolayer growth. (FIGS. 1, 2). Cells have light refractory or round or polygonal cell borders. The cells will also form fluid filled domes with cells exhibiting the currently described ES morphology. The doubling rate of these cells is about 18-36 hours. These characteristics differ little from those reported for mouse embryonic stem cells, but do differ significantly from those reported by Evans. (FIG. 1).

Similarities of swine to mouse embryonic stem cells include that the nucleus to cytoplasmic ratio is approximately 85:15. The nucleus is round and contains several prominent nucleoli. Cell size varies somewhat lines isolated, but most of the stable lines consist of cells with diameters in the range of 8-15 microns.

In Table 2, the differences between the purported ES swine cells of Evans and those of the present invention are set forth. Also, the similarities between the swine cells disclosed herein and the ES cells of mice are described.

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Size	11-12 $\mu$ m	"larger than those of the mouse"	8-15 $\mu$ m
Shape	round	round	round
Monolayer colonies	no	yes	no
Distinct individual cells can be identified	no	yes	no
Cytoplasm (% of cell vol)	25%	small	10-25%
Nucleus (% cell vol)	75%	large (no data)	75-90%
Number of nucleoli	2-4	2-4	2-4
Teratocarcinoma production in SCID mouse	yes <sup>b</sup>	?	yes
State of embryo cultured	3.5 d blasto- cysts	6.5-11 d hatched blastocysts	7.5-10 d hatched blastocysts

<sup>a</sup> Evans relates "a variety of sizes" (column 10 of Evans patent), and sizes varying among cell lines.

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